Claims

1. A fluorogenic protease substrate comprising a peptide doubly labelled via thiol groups of the peptide with an alkyleneamidotetramethylrhodamine (alkyleneamido-TMR) group.

2. A fluorogenic protease substrate according to claim 1, which is doubly labelled with the same alkyleneamido-TMR group.

3. A fluorogenic protease substrate according to claim 2, wherein the alkyleneamido-TMR group is a methyleneamido-TMR group.

4. A fluorogenic protease substrate according to claim 2 or claim 3, wherein the peptide is doubly labelled with an isomeric form of the alkyleneamido-TMR group that is at least 90% pure with respect to other isomeric forms of the alkyleneamido-TMR group.

5. A fluorogenic protease substrate according to claim 4, wherein the alkyleneamido-TMR group is 5-alkyleneamido-TMR or 6-alkyleneamido-TMR.

6. A fluorogenic protease substrate according to claim 4 or claim 5, wherein the level of purity is at least 95%.

7. A fluorogenic protease substrate according to claim 6, wherein the level of purity is at least 98%.

8. A fluorogenic protease substrate according to any preceding claim, which contains one or more protease recognition sequences for one or more proteases of interest.

9. A fluorogenic protease substrate according to claim 8, wherein the protease recognition sequence is 2 to 8 amino acids in length.

10. A fluorogenic protease substrate according to any preceding claim, which is 4-20 amino acids in length, optionally excluding any terminal cysteine residues.

11. A fluorogenic protease substrate according to claim 10, which is 4-12 amino acids in length.

12. A fluorogenic protease substrate according to claim 11, which is 6-10 amino acids in length.

13. A fluorogenic protease substrate according to any preceding claim, which does not adopt a well-defined conformation, as determinable by NMR spectroscopy.

14. A fluorogenic protease substrate according to any preceding claim, wherein the alkyleneamido-TMR groups are attached to the peptide via cysteine residues.

15. A fluorogenic protease substrate according to claim 14, wherein the cysteine residues are C- and N-terminal cysteine residues.

16. A fluorogenic protease substrate according to claim 14, wherein the cysteine residues are internal, and the

peptide is susceptible to protease cleavage between the cysteine residues.

17. A fluorogenic protease substrate according to any preceding claim, wherein the peptide contains exactly two cysteine residues.

18. A method for producing a fluorogenic protease substrate as defined in any preceding claim, the method comprising reacting an unlabelled peptide containing two thiol groups with haloalkylamido-TMR.

19. A method according to claim 18, wherein the halogen atom of the haloalkylamido-TMR is iodine.

20. A method according to claim 19, wherein the haloalkylamido-TMR is iodoacetamidotetramethylrhodamine (IATR).

21. A fluorogenic protease substrate comprising a peptide doubly labelled with the same rhodamine derivative, where the two labels, and their linkages to the peptide, are substantially isomerically identical.

22. A fluorogenic protease substrate according to claim 21, wherein the label is linked to the peptide via thiol groups on the peptide.

23. A fluorogenic protease substrate according to claim 21 or claim 22, wherein the rhodamine derivative is a tetramethylrhodamine derivative.

24. A method for assaying protease activity in a sample, the method comprising bringing into contact the sample and a fluorogenic substrate as defined in any one of claims 1 to 17 and 21 to 23 under conditions suitable for protease activity, and determining whether an increase in fluorescence results.

25. A method according to claim 24, wherein fluorescence is determined for the substrate before and after contact with the sample.

26. A method according to claim 24 or claim 25, wherein the step of contacting the sample and the substrate occurs at a pH of between about 5 and 10.

27. A method according to any one of claims 24 to 26, wherein the sample is a tissue sample, or other sample containing intact cells.

28. A method according to any one of claims 24 to 27, wherein the method is for assaying activity of a known protease, and wherein the substrate comprises the recognition sequence for that protease.

29. A kit for use in a method of assaying protease activity, the kit comprising a fluorogenic protease substrate as defined in any one of claims 1 to 17 and 21 to 23 and a standard protease composition for calibration of the assay.

30. A kit according to claim 29, wherein the fluorogenic protease substrate is immobilised.

31. A solid support having immobilised thereon a fluorogenic protease substrate as defined in any one of claims 1 to 17 and 21 to 23.

32. A solid support according to claim 31, bearing different said substrates respectively immobilised at different locations of the support.